EXHIBIT A

Attorney's Docket No.: 12875-002001 / 0643-5299US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Wei-Yu Lo et al.
Serial No. : 09/778,516
Filed : February 7, 2001
Title : LAC SHUTTLE VECTORS

Art Unit : 1633
Examiner : Unknown

BOX SEQUENCE
Commissioner for Patents
Washington, D.C. 20231

RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE AND/OR AMINO ACID SEQUENCES

In response to the communication dated May 10, 2001 (copy enclosed), applicants submit herewith a Sequence Listing in computer readable form as required by 37 CFR §1.824. In addition, applicants submit an initial Sequence Listing as required under 37 CFR §1.823(a) and a statement under 37 CFR §1.821(f).

Applicants respectfully request entry of the paper copy and computer readable copy of the Sequence Listing filed herewith for the instant application. Please insert the Sequence Listing following the Oath/Declaration. Furthermore, applicant requests entry of the following amendments.

In the specification:

Replace the paragraph beginning at page 4, line 21, with the following rewritten paragraph:

--FIG. 4 is a diagram showing the constructs of the pCLP7 and pCLP8. Nucleotides 687 to 735 of SEQ ID NO:1 and nucleotides 736 to 784 of SEQ ID NO:1 are shown below.--

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

July 10, 2001
Date of Deposit

Signature

Jennifer H. Payne
Typed or Printed Name of Person Signing Certificate

Replace the paragraph beginning at page 14, line 19, with the following rewritten paragraph:

--The β-galactosidase gene was amplified from chromosomal DNA of *Lactobacillus delbrueckii* (subsp. *bulgaricus*) by polymerase chain reaction (PCR). The PCR amplification consisted of 0.075 units *Pfu Turbo*™ DNA polymerase (STRATAGENE®, La Jolla, CA), 1 μM each of forward (5'-aagctcatgaTTGGCAGCCAGTCTCCGGGC-3' (SEQ ID NO:3) and reverse primers (5'-gacctcatgaACCGTCGCTAGCGACACGCC-3' (SEQ ID NO:4). PCR was carried out in 4 stages: (i) 95°C for 5 min; (ii) 94°C for 30 sec, 54°C for 30 sec, 72°C for 3 min, x 30 cycles; (iii) 72°C for 10 min; and (iv) hold at 4°C. The amplified DNA product was estimated by 0.8% agarose electrophoresis and ethidium bromide stain, followed with purification by GENECLEAN III kit (Bio 101, La Jolla, CA). The purified 3 kb β-galactosidase DNA fragment was ligated into *EcoRV* site of pcDNA3 vector (INVITROGENE). The ligation mixture was transformed into *E. coli* strain DH5α. The blue-color clones containing the plasmid bearing β-galactosidase gene were selected from X-gal/Amp LB agar plate.--

Replace the paragraph beginning at page 15, line 12, with the following rewritten paragraph:

--The plasmid pVA838 obtained from CCRC (Hsinchu, Taiwan) was used as template for cloning of (Em'P) DNA fragment via PCR. The PCR amplification consisted of 0.075 units *Pfu Turbo*™ DNA polymerase (STRATAGENE®), 1 μM each of forward (5'-TTAACGATCGTTAGAAGCAAACTTAAGAGTG-3' (SEQ ID NO:5) and reverse primers (5'-TTAACGATCGATGTAATCACTCCTTCT-3' (SEQ ID NO:6). PCR was carried out in 4 stages: (i) 95°C for 5 min; (ii) 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec, x 30 cycles; (iii) 72°C for 10 min; and (iv) hold at 4°C. The amplified DNA product was estimated by 1% agarose electrophoresis and ethidium bromide stain, followed with purification by phenol/chloroform extraction and ethanol precipitation. The purified 120 bp Em'P DNA fragment was ligated into the pCRII vector (INVITROGENE). These clones bearing the pCRII/Em'P plasmid were selected from X-gal/Amp LB agar plate as white colonies and further checked by PCR and restriction enzyme analysis.--

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REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. I hereby state, as required by 37 C.F.R. §1.821(g), that the enclosed submission includes no new matter.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 7-9-01

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"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 4, line 21, has been amended as follows:

FIG. 4 is a diagram showing the constructs of the pCLP7 and pCLP8. Nucleotides 687 to 735 of SEQ ID NO:1 and nucleotides 736 to 784 of SEQ ID NO:1 are shown below.

Paragraph beginning at page 14, line 19, has been amended as follows:

The β-galactosidase gene was amplified from chromosomal DNA of *Lactobacillus delbrueckii* (subsp. *bulgaricus*) by polymerase chain reaction (PCR). The PCR amplification consisted of 0.075 units *Pfu Turbo*™ DNA polymerase (STRATAGENE®, La Jolla, CA), 1 μM each of forward (5'-aagctcatgaTTGGCAGCCAGTCTCCGGGC-3'; SEQ ID NO:3) and reverse primers (5'-gacctcatgaACCGTCGCTAGCGACACGCC-3'; SEQ ID NO:4). PCR was carried out in 4 stages: (i) 95°C for 5 min; (ii) 94°C for 30 sec, 54°C for 30 sec, 72°C for 3 min, x 30 cycles; (iii) 72°C for 10 min; and (iv) hold at 4°C. The amplified DNA product was estimated by 0.8% agarose electrophoresis and ethidium bromide stain, followed with purification by GENECLEAN III kit (Bio 101, La Jolla, CA). The purified 3 kb β-galactosidase DNA fragment was ligated into *EcoRV* site of pcDNA3 vector (INVITROGENE). The ligation mixture was transformed into *E. coli* strain DH5α. The blue-color clones containing the plasmid bearing β-galactosidase gene were selected from X-gal/Amp LB agar plate.

Paragraph beginning at page 15, line 12, has been amended as follows:

The plasmid pVA838 obtained from CCRC (Hsinchu, Taiwan) was used as template for cloning of (Em^{r}P) DNA fragment via PCR. The PCR amplification consisted of 0.075 units *Pfu Turbo*™ DNA polymerase (STRATAGENE®), 1 μM each of forward (5'-TTAACGATCGTTAGAAGCAAACTTAAGAGTG-3'; SEQ ID NO:5) and reverse primers (5'-TTAACGATCGATGTAATCACTCCTTCT-3'; SEQ ID NO:6). PCR was carried out in 4 stages: (i) 95°C for 5 min; (ii) 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec, x 30 cycles; (iii) 72°C for 10 min; and (iv) hold at 4°C. The amplified DNA product was estimated by 1%

agarose electrophoresis and ethidium bromide stain, followed with purification by phenol/chloroform extraction and ethanol precipitation. The purified 120 bp Em^{r}P DNA fragment was ligated into the pCRII vector (INVITROGENE). These clones bearing the pCRII/Em^{r}P plasmid were selected from X-gal/Amp LB agar plate as white colonies and further checked by PCR and restriction enzyme analysis.